Factors Affecting Dispersal of *Mucor piriformis* in Pear Orchards and into the Packinghouse

THEMIS J. MICHAILIDES, Postdoctoral Research Associate, and R. A. SPOTTS, Associate Professor, Department of Botany and Plant Pathology, Oregon State University, Mid-Columbia Agricultural Research and Extension Center, Hood River, OR 97031

ABSTRACT

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Mucor piriformis is one of the major causes of postharvest decay of pear fruit in the Pacific Northwest. Propagules of *M. piriformis* numbering 6–3,381/g of dry soil were found in soils collected from five pear orchards 1 mo before harvest; however, *M. piriformis* was absent in samples of leaf, fruit, and air collected during harvest. At harvest 2.5–5% of decayed fruits on the orchard floor were infected with *M. piriformis*. Two months later, fallen fruits decayed by *M. piriformis* increased to 23–50%, and propagules of *M. piriformis* in the soil increased to 365–6,832/g of dry soil. Soil adhering on the picking bins had 1,042–8,333 fungal propagules per gram of dry soil and may serve as an inoculum source of postharvest infections of pear fruits.

In the Pacific Northwest fruit industry, infection of Anjou pear (*Pyrus communis* L.) and apple (*Malus sylvestris* Mill.) fruits by *Mucor piriformis* Fischer has produced serious losses during cold storage (2). Although some Mucor rot occurs every year, severe disease outbreaks may be related to environmental factors as well as improper handling of the fruit during harvest and packaging. *M. piriformis* survives in the soil primarily as sporangiospores (6), and populations of propagules are greater in the upper 5 cm of soil than at 5–30 cm (13).

In packinghouses, pear fruits are removed from field bins by immersion dumping (1). Although dump-tank water commonly contains either chlorine or sodium ortho phenylphenate and a flotation salt, studies have shown that water samples taken from dump tanks in packinghouses contained viable propagules of *M. piriformis* and *Penicillium expansum* Link ex Thom (1,11,12).

This study was conducted to investigate factors affecting dispersal of propagules of *M. piriformis* in the orchard and from the orchards into the packinghouse. An abstract of this work has been published (7).

Present address of first author: Department of Plant Pathology, University of California, Davis 95616.

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MATERIALS AND METHODS

Soil and debris sampling. Three composite soil samples (80–100 g each) were collected with a soil-tube sampler (2 cm i.d.) in five orchards in the Hood River Valley, Oregon, 1 mo before and 2 mo after harvest. The five orchards were located at four distinct geographic locations throughout the valley (Eastside, Westside, Parkdale [South], and Hood River [North]). Soil was taken at depths of 0–5 cm from four sides of three pear trees, and samples were composited. The soil dilution plate technique was used to determine numbers of fungal propagules in soil. The plates were prepared by spreading evenly 0.1 ml of a 1:100–1:1,000 soil dilution in sterile distilled water onto the surface of each of three potato-dextrose agar plates acidified to pH 3.5 ± 0.1 with 2.5 ml of lactic acid (25%) per liter (APDA).

From each orchard, 20–30 g of debris (litter of orchard surface excluding pear mummies) was removed from the soil surface at three locations, and samples were composited. Debris was dried at 20 C, then ground to a particle size of 2–3 mm^2. Two grams of debris was placed in 50 ml of sterile distilled water and mixed for 2 hr at 100 rpm in a rotary shaker. After mixing and 0.5 hr of settling, 0.1 ml of suspension was plated on APDA. All plates were incubated 20–24 hr at 20 C and fungal colonies counted by observing the plates through transmitted light. Propagules of *M. piriformis* were expressed per gram of oven-dry soil or dry debris. Data of propagules of *M. piriformis* from soil and debris samples were transformed to log + 1 to equalize variances and analyzed by ANOVA with the Statistical Analysis Systems (SAS [8]); mean separation was conducted by using Tukey's studentized range (HSD) test.

Soil sampling from harvest bins. Five- to 10-g soil samples were scraped from each of 20 random harvest bins in a pear orchard (Parkdale no. 33) during a commercial harvest operation and assayed for *M. piriformis* propagules on APDA plates with the dilution technique described before.

Fruit, leaf, and orchard air sampling. Three separate samples, each consisting of five fruits and 25 leaves, were collected from three pear trees in each orchard where soil samples were taken. After collection, the samples were washed in 50 ml of sterile distilled water and 0.1 ml of washing water was plated on APDA. Colonies of *M. piriformis* were recorded after 20–24 hr of incubation of the plates at 20 C and again after 2–3 days for other fungal colonies.

For air sampling, two air samplers consisting of a sequential valve assembly (115V/50–60 Hz) (Gelman, Richardson, TX) were placed close to the bin collection and loading area during a commercial harvesting operation and used as spore traps. During an 8-hr operation, orchard air was forced through Millipore (0.45-μm pore size) filters (Millipore Corporation, Bedford, MA), and the valve was designed to shift the flow to each of the four ports in sequence every 2 hr. Airflow was measured with a Series 500 flow meter (F. W. Dwyer Mfg. Co., Michigan City, IN) and adjusted to 1.135 m^3/hr. After sampling, filters were removed and placed in contact with the surfaces of three APDA plates per filter for 10 sec each. Plates were incubated at 18–20 C for 24 hr, colonies were counted and reincubated at room temperature (21 ± 2 C) for 2 days, and final counts of colonies of *M. piriformis* were made.

Infection of fruit on orchard floor. Twenty to 30 decaying pear fruits on the orchard floor were collected from each of the five orchards at harvest (mid-

September). Fruit tissues were plated on APDA plates and incubated at 20 C for 3–4 days. Only *Mucor* spp. and *Botrytis cinerea* Pers.: Fr. were identified. In addition, two boxes of apparently sound pears were collected from the orchard floor from a 0.2-ha area surrounding the soil-sampling area. Fruits were stored at −1 C and decay recorded after 6 mo. To determine the frequency of decay organisms on fallen pear fruit after harvest, 100 pears were examined on 19 and 20 November in each of the five orchards. *M. piriformis, B. cinerea,* and *P. expansum* were identified visually by characteristic sporulation on infected fruit, whereas all other fungi were designated as "other rot fungi." ANOVA was conducted with SAS (8), and mean separation with Tukey's test.

In Parkdale orchard 33, incidence and spatial distribution of *M. piriformis* decay on fallen fruit was studied. A Bartlett pear tree was not harvested, and all of its fruit dropped to the ground by 4 October. Fruit positions were mapped and recorded for decay caused by *M. piriformis* on 4, 9, and 15 October and 1 November.

Cold storage of fruit harvested from trees. To determine development of decay in cold storage, 10 boxes consisting of 75–115 fruits per box were hand-harvested from each of the five orchards, i.e., two boxes per each of five pear trees (including the three trees previously sampled for fruit and leaves). Fruits were placed in polyethylene-lined boxes, stored at −1 C, and examined after 6 mo for number of decayed fruit. ANOVA and mean calculations were conducted with the SAS (8), and mean comparisons with Duncan's multiple range test.

RESULTS

Soil and debris sampling. Populations of *M. piriformis* propagules in soils collected before pear harvest ranged from 6 to 3,381/g of dry soil. Two Westside orchards had the lowest propagule numbers, whereas those in Eastside, Hood River, and Parkdale contained 2,000/g of dry soil or more (Table 1). Sampling of soil 2 mo after harvest revealed propagule levels ranging from 365 to 6,832/g of dry soil. The effect of sampling time was significant (F = 33.33, df = 1, P <0.01). The Westside orchards again had the lowest counts (Table 1). Propagules of *M. piriformis* in debris ranged from 0 to 133/g and represented 0–6% of *M. piriformis* populations found in the upper 5 cm of soil (Table 1). Propagules of *M. piriformis* were not found in debris samples collected from the Westside orchards.

Soil sampling from harvest bins. Nineteen of 20 samples had *M. piriformis* propagule counts ranging from 1,042 to 8,333/g of dry soil, and of these, 14 samples had <5,000, four had 5,208, and one 8,333/g of dry soil.

Fruit, leaf, and orchard air sampling. No *M. piriformis* was found in washings from any of the fruit or leaf samples taken from the trees at harvest. However, *P. expansum, B. cinerea,* and *Cladosporium* spp. were washed from fruit and leaf surfaces. In addition, propagules of *M. genevensis* Lendner and occasionally *M. plumbeus* Bonorden were recovered from leaf surfaces.

M. piriformis was not found in any air samples, but propagules of *B. cinerea, P. expansum, Alternaria alternata* (Fr.) Keissler, and *Cladosporium herbarum* (Pers.) Link ex Gray were recovered from Millipore filters through which orchard air was passed.

Infection of fruit on orchard floor. Most of the fruits that fell on the ground during harvest (mid September) were rotted by *B. cinerea* (15–55%) and *Rhizoctonia solani* Kühn, *A. alternata, P. expansum,* and *Phytophthora cactorum* (Leb. & Cohn) Schroet. (overall 40–83% of decaying fallen fruit). Fruit decay attributed to *M. piriformis* was found only in the orchards at Parkdale (no. 33) (3%) and Hood River (no. 129) (5% of decaying fruit). Healthy fruit collected from the ground at harvest showed 0–3% decay caused by *M. piriformis* and 37–83% total decay (Mucor, Botrytis, Gloeosporium, and other rots) after 6 mo of storage at −1 C (Table 2).

Two months after harvest (19–20 November), 23–50% of the fallen fruit left on the ground showed evidence of *M. piriformis* infections. Two to 20% of pears were infected by *B. cinerea* and 6–14% by other fungi (Table 3). The percentage of fruits decaying with *M. piriformis* was significantly higher than that of fruits decaying with *B. cinerea* or other rot fungi (F = 115.78, df = 2, P <0.01). In the Westside orchards, the percentage of fruit decayed by *M. piriformis* was lower than in other orchards.

Development and spread of *M. piriformis* decay among fallen pears was relatively fast. For instance, on 4 October, three pears were decaying with *M. piriformis* (Fig. 1A). Within 5 days, seven more pears had decayed, and by 15 October, a total of 46 pears in a population of 110 pears examined showed decay and sporulation of *M. piriformis* (Fig. 1B,C). By 1 November, all pears except one were decaying (Fig. 1D) and sporulation of *M. piriformis* was

Table 1. Propagule levels of *Mucor piriformis* in soil and debris from five pear orchards 1 mo before and 2 mo after fruit harvest (Hood River Valley, Oregon)

Orchard location and number	Propagules of *M. piriformis* per gram[x]		
	Before harvest		After harvest
	Debris	Soil[y]	Soil[y]
Parkdale (33)	33 ab[z]	3,381 a	4,668 a
Hood River (129)	83 ab	2,700 a	4,249 a
Eastside (120)	133 a	2,090 a	6,832 a
Westside B (97)	0 b	33 b	735 b
Westside A (95)	0 b	6 b	365 b

[x] Propagule levels are the average of 27 replicate dilution APDA plates (three composite soil samples per orchard).
[y] The ANOVA was performed after a log + 1 transformation of the data and indicated that the "after harvest" level of propagules in soil was significantly higher than the "before harvest" level (F = 33.33, df = 1, P <0.01).
[z] Numbers followed by the same letter within columns are not significantly different using Tukey's studentized range (HSD) test (P = 0.05).

Table 2. Decay of pear fruit harvested from trees and collected from the ground at harvest in five orchards and stored at −1 C for 6 mo

Orchard location and number	Decay (%) of fruit			
	Mucor piriformis		Total decay[w]	
	Tree[x]	Ground[y]	Tree[x]	Ground[y]
Parkdale (33)	1 a[z]	3 a	9 b	49 ab
Hood River (129)	1 a	1 a	13 ab	83 a
Eastside (120)	0 a	1 a	15 ab	70 ab
Westside B (97)	2 a	0 a	8 b	58 ab
Westside A (95)	0 a	1 a	24 a	37 b

[w] Total decay included Mucor, Botrytis, Gloeosporium, and other rots. The ANOVA was performed after an arc sine transformation of the data and indicated that the total decay of fruits collected from the ground was significantly higher than that of fruits harvested from the trees (F = 175.55, df = 1, P <0.01).
[x] Percentage of decayed fruits harvested from the trees is based on 10 replicate boxes (75–115 fruits per box) per orchard.
[y] Percentage of decayed fruit collected from the ground is based on two replicate boxes (85–100 fruits per box) per orchard.
[z] Numbers followed by the same letter within columns are not significantly different according to Duncan's multiple range test (P = 0.05).

evident on all affected fruits. Most decayed pears showed signs of insect and/or bird damage (Fig. 2A–C).

Cold storage of fruits. Decay of pear fruits harvested from trees and stored 6 mo at −1 C varied from 8 to 24% among orchards. However, only up to 2% decay was caused by *M. piriformis* (Table 2). Although there were no significant differences among orchards in percentage of decay from *M. piriformis* of fruit harvested from the trees or the ground, total decay of fruit harvested from the trees was significantly less than the percentage of total decay of fruit harvested from the ground ($F = 175.55$, df = 1, $P < 0.01$). *B. cinerea* caused more decay in storage than any other fungus.

DISCUSSION

In the Hood River Valley, it is a common practice to interplant Bartlett and Anjou pears in alternating rows for better pollination. Bartlett trees are harvested 15–20 days earlier (middle of August to beginning of September) than the Anjou. During harvest of Anjou pears (mid-September), Bartlett fruit already fallen on the ground decays, primarily from *B. cinerea*. In mid-November, about 2 mo after Anjou harvest, when temperatures are low and unfavorable for other fungi, a large percentage of fallen fruit becomes infected with *M. piriformis*. Although optimum temperature for growth of *M. piriformis* is 20 C (2,4), it can grow and sporulate extensively at −1 to 0 C (4,5). During November, the fungus was also observed sporulating on fallen leaves and on soil close to decaying fruits. The significant increase of fungal propagules after harvest may be related to inoculum produced by rotting fruit. Sporangiospores of *M. piriformis* produced on rotted fruit are easily dislodged by rain and enter the soil matrix. These spores have been shown to be the long-term survival structures of the fungus (6).

In the orchard, the efficient spread of *M. piriformis* decay among fruit lying on the ground is indicated by the rapid increase of pears decaying with *M. piriformis* within a relatively short time

Table 3. Fungi recorded in pear (Anjou) fruit on the ground in five pear orchards 2 mo after fruit harvest

Orchard location and number	Percentage of fruit decaying with[y]		
	Mucor piriformis	*Botrytis cinerea*	Other rot fungi
Parkdale (33)	41 ab[z]	6 bc	6 a
Hood River (129)	39 ab	2 c	13 a
Eastside (120)	50 a	10 b	6 a
Westside B (97)	23 b	10 b	14 a
Westside A (95)	24 b	20 a	13 a

[y] Percentage of decay was determined by recording 100 random pears (four replicates of 25 fruits) on the orchard ground in a 0.2-ha area at the place where the soil samples were taken.

[z] Numbers in each column followed by the same letter are not significantly different according to Tukey's studentized range (HSD) test ($P = 0.05$). The ANOVA was performed after an arc sine transformation of the data and indicated that percentage of fruits decayed from *M. piriformis* was significantly higher than that of fruits decayed from *B. cinerea* or other rot fungi ($F = 115.78$, df = 2, $P < 0.01$).

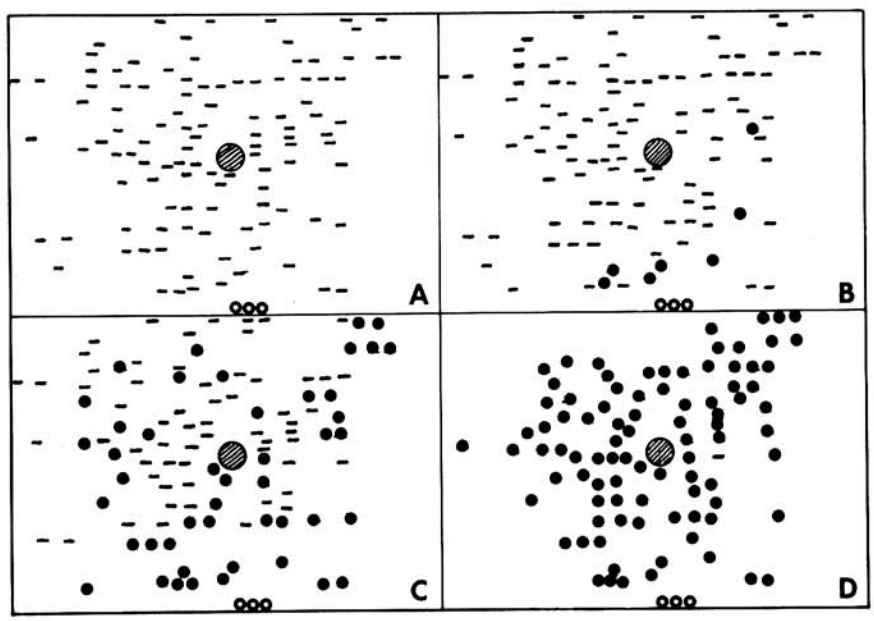

Fig. 1. Pattern of successive spread of *Mucor piriformis* decay among pears (Bartlett) dropped on the ground: **(A)** on 4 October, **(B)** on 9 October, **(C)** on 15 October, and **(D)** on 1 November 1984.

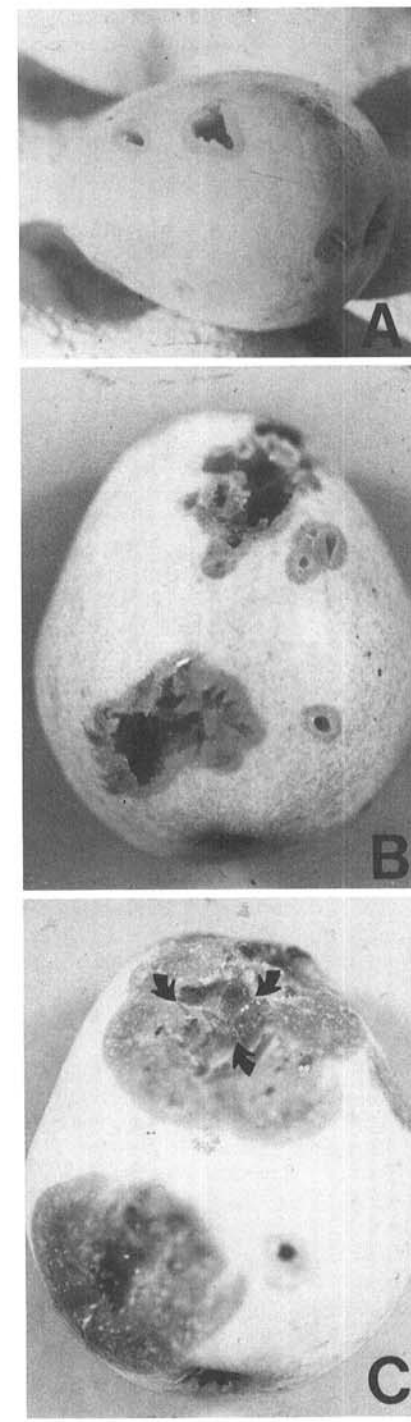

Fig. 2. Anjou pears with signs of insect and/or bird damage: **(A)** collected in the field and after **(B)** 1 day and **(C)** 2 days of incubation at 20 C. Arrows delimit area of sporulation of *Mucor piriformis*.

span. The spatial pattern was entirely random. Feeding signs of insects, birds, and/or other animals (probably mice) were evident (Fig. 2), and insects were observed feeding on pear fruit during fruit sampling. Additional evidence that insects and other animals contribute to disease spread is that decay was commonly initiated on the upper fruit surface and not that touching the soil. Wind dispersal is not considered a means for dissemination because sporangiospores of *M. piriformis* are embedded in a mucilagenous matrix (4). In *Mucor* species, this mucilage dries under low humidity, leaving the spores firmly cemented to one another and to the columella so that even strong air currents fail to dislodge the spores (3).

Incidence of decay of fruit harvested from trees and stored for 6 mo did not correlate with *M. piriformis* propagule populations occurring in orchard soils ($r = 0.14$, needed $r = 0.80$ at $P = 0.05$). This was expected because *M. piriformis* was not recovered from surfaces of leaves and fruit on the trees or in samples of orchard air. In addition, survival of *M. piriformis* on fruit surfaces on the tree is reported to be shorter than that of *B. cinerea* or *P. expansum* (10). However, washings from leaves and fruits and filters from spore traps contained an abundance of *B. cinerea, P. expansum,* and *Cladosporium* spp., all of which are known to constitute the major component of the air spora in general (3).

The results of our study do not account for the small percentage (1–3%) of decay from *M. piriformis* in pears collected from the trees and the ground and stored for 6 mo. It is possible that a few propagules of *M. piriformis* were on the pears and our techniques of washing the pears and plating the washings were probably not sensitive enough to detect them. It is also possible that there was some contamination of fruit from the wooden harvest boxes. The identical percentages of decay from *M. piriformis* on fruits collected from the trees and from the ground can be explained by the fact that *M. piriformis* requires wounds for infection, and particular care was used in collecting all fruit samples.

Our evidence indicates that soil is the primary source for contamination of pears by *M. piriformis*. The fact that only sound-appearing pears were selected from the orchard floor may help to explain the lack of correlation of the source (orchard) with decay incidence realized after prolonged storage. The decay of pears collected from the ground caused by various fungi (total decay, Table 2) was significantly higher than that of fruit harvested from the trees, indicating that dirt brought in with the fruit in storage contributed to higher levels of total decay.

During harvest, bottoms of bins are covered with soil and debris. In packinghouses, immersion dumping is a common practice (1). Dump-tank water is thus contaminated with the dirt and debris brought in with the bins. Soil samples taken by scraping the bottoms of harvest bins had high levels of propagules of *M. piriformis* (average from 20 bins = 2,865/g of dry soil). Even though packinghouses routinely use chlorine or other chemicals in dump-tank water to reduce fungal propagules, *M. piriformis* and *P. expansum* are still commonly isolated from samples of dump-tank water (1,2,12). Soil and debris in dump-tank water lodge in puncture wounds, usually created by the fruit stems during harvest. The incidence of decay of pears caused by *M. piriformis* determined in this study is low compared with that reported by packinghouse operators (R. A. Spotts and T. J. Michailides, *unpublished*) and can be explained by the different method of handling. All fruits used in our experiments were harvested by hand and immediately stored without going through the process used in commercial packinghouse operations. Smith et al (9) also suggested that contamination of peach fruit occurs in packinghouse operations and showed that fruit picked by hand had less decay than that commercially harvested, graded, and hydrocooled.

Conclusion. Our data suggest that orchard sanitation practices such as removing fallen fruit (to minimize buildup of *M. piriformis* propagules resulting from extensive sporulation on infected fruit), avoiding harvesting during wet soil conditions, and washing soil from fruit bins before the dump-tank operation should be done. In addition, fruit dropped on the ground should never be collected and placed in harvest bins.

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